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(54) Title: ASSAY

(57) Abstract: The present invention provides an assay device for detection of an analyte which is a member of a specific binding partner in a sample, the assay device comprising a sample application zone, a preabsorbing zone and a specific binding zone. The device can provide a HSV-2 specific assay by preabsorbing HSV-1 antibodies in the preabsorbing zone.

1 **Assay**

2

3 The present invention relates to an improved ligand -
4 receptor assay and a method of enhancing the
5 specificity of assays which involve detection of an
6 analyte which constitutes one member of a pair of
7 specific binding partners (SBP) by detection of its
8 binding to the other member of said pair.

9

10 Assay based tests involving SBP are in widespread use
11 by healthcare providers and the public for diagnosis of
12 a variety of conditions.

13

14 The present invention seeks to enhance the specificity
15 of assays for the presence of ligands which are one
16 member of a pair of SBP by effecting the removal of
17 potentially cross-reactive ligands prior to analysis of
18 the first ligand.

19

1 In the field many assays utilise one member of a pair
2 of SBP bound to a solid phase with which a sample
3 suspected of containing the second member of the pair
4 of SBP is then interacted. Any second member of the
5 SBP which is bound to the first member of the SBP
6 which in turn is bound to the solid-phase antigen is
7 then detected to constitute a positive result in the
8 test.

9
10 Many assays seek to determine the presence or absence
11 of one member of a pair of SBP by detecting the
12 binding of this first member to its binding partner.
13 The disadvantage of this method is that the sample
14 utilised in the assay may also contain other ligands
15 which are potentially capable of binding to the
16 second member of the pair of SBP. For example, an
17 assay may involve the detection of antibodies to a
18 particular antigenic species possibly in the presence
19 of other antibodies which are potentially cross
20 reactive with the antigenic species. Unless the
21 binding of the member of the pair of SBP to which the
22 member of the pair of SBP suspected of being present
23 in the sample binds during the assay is highly
24 specific to the member of the pair of SBP suspected
25 of being present in the sample the presence of
26 potentially cross-reactive ligands can compromise the
27 specificity of such assays, leading to the occurrence
28 of false positive results and, potentially, incorrect
29 or inappropriate clinical management of a patient
30 arising from use of such a false result. Several
31 methods have been employed to enhance the specificity

1 of the members of pairs of SBP used in these tests.
2 These include use of synthetic peptide/non-peptide
3 mimics of the member of the pair of SBP or, in the
4 case where the member of the pair of SBP is a
5 protein, use of recombinant protein analogues of the
6 member of the pair of SBP. The potential
7 disadvantage of such SBP analogues is that they may
8 not be identical to the SBP encountered by the
9 patient. There is, therefore, a potential for assays
10 using such artificial SBP to elicit falsely negative
11 results and, potentially, incorrect or inappropriate
12 clinical management of a patient arising from use of
13 such a false result.

14

15 It is an object of the present invention to provide
16 an improved binding assay.

17

18 The present invention describes an assay format
19 wherein a sample suspected of containing one member
20 of a pair of SBP, the "analyte", is first exposed to
21 one or more moieties which are capable of binding
22 members of other SBP which could potentially cross-
23 react with the other member of the pair of SBP to
24 which the analyte binds, before then being exposed to
25 said second member of the pair of SBP of which the
26 analyte is the first member. Analyte bound to its
27 specific binding partner is then detected using a
28 suitably-labelled second binding partner which binds
29 to the analyte at a site other than the binding site
30 for the analyte and the first SPB.

31

1 Preferably, such an assay shall be composed of a
2 membrane capable of conducting fluid flow, this
3 membrane comprising of a sample application zone, a
4 fluid absorbent zone, a line or lines of immobilised
5 analyte antigens and one or more lines of immobilised
6 receptor moieties which function as preabsorbing
7 groups.

8
9 Preferably the line or lines of said immobilised
10 preabsorbing groups and analyte antigens are
11 interposed between the sample application zone and
12 the fluid absorption zone.

13
14 Fluid flow along the membrane from said sample
15 application zone to towards the fluid absorption zone
16 is preferably facilitated by means of capillary
17 action.

18
19 Preferably such movement of the sample will result in
20 the analyte antibodies and cross-reacting antibodies
21 to first contact the preabsorbing groups and
22 subsequently the analyte antigens.

23
24 Also preferably, the line or lines of immobilised
25 analyte antigens are interposed between the line or
26 lines of immobilised preabsorbing groups and the
27 fluid absorption zone.

28
29 The application of such lines of immobilised moieties
30 should be such that lateral fluid flow along the

1 membrane causes all the components present in the
2 fluid to contact the lines of immobilised moieties.

3
4 Samples assayed by means of the present invention may
5 be selected from, but not limited to whole blood,
6 serum, plasma, interstitial fluid, semen, seminal
7 plasma, urine and saliva.

8
9 Detection of analyte antibodies, which are bound to
10 the membrane by complexation with their respective
11 analyte antigens is by means of a suitably labelled
12 reagent, which is capable of binding to said SBP
13 complex, wherein this labelled reagent can be
14 detected.

15
16 The device can similarly detect analyte antigens by
17 complexation with immobilised antibodies.

18
19 Such labelling reagent may be selected from the group
20 consisting of, but not limited to, those which
21 dispose a visually detectable moiety at the site of
22 binding, those which dispose a fluorescent moiety,
23 whereby such a moiety can be visualised either by
24 fluorescence spectrometry or visually upon
25 application of light at an appropriate wavelength to
26 cause fluorescence of the flurophore employed.

27
28 Detection said labelled reagent may be by means a
29 catalytic moiety which is attached to the labelled
30 reagent, wherein the catalytic moiety is subsequently
31 exposed to a substrate wherein a visually

1 discernible, fluorescent, or chemiluminescent product
2 is generated by the action of said catalytic moiety.

3

4 Colloidal gold and any combination of coloured latex
5 can be used for labelling.

6

7 A general embodiment of the invention may comprise

8

9 1. Forming an assay for the detection of antibodies
10 ("analyte antibodies") which may be present in a
11 sample; such antibodies being the specific binding
12 partners of one or more particular antigens
13 ("analyte antigens"), such an assay being composed
14 of a membrane capable of conducting fluid flow and
15 incorporating a sample application zone, a fluid
16 absorbent zone, a line or lines of immobilised
17 analyte antigens and one or more lines of
18 immobilised receptor moieties ("preabsorbing
19 groups") capable of binding antibodies ("cross-
20 reacting antibodies") which may be present in the
21 sample and which are potentially capable of
22 binding to sites on the analyte antigens other
23 than the site or sites on the analyte antigens
24 which bind the analyte antibodies and such that
25 the line or lines of immobilised preabsorbing
26 groups and analyte antigens are interposed between
27 the sample application zone and the fluid
28 absorption zone, and the line or lines of
29 immobilised analyte antigens are interposed
30 between the line or lines of immobilised
31 preabsorbing groups and the fluid absorption zone.

- 1 Lines of immobilised moieties are applied to the
2 membrane such that lateral fluid flow along the
3 membrane causes all components present in the
4 fluid to contact the lines of immobilised
5 moieties.
- 6 2. Applying a sample which may be one of a group
7 which includes but is not limited to whole blood,
8 serum, plasma, interstitial fluid, semen, seminal
9 plasma, urine, or saliva to the sample application
10 zone on the membrane such that fluid flow along
11 the membrane by capillary action from the sample
12 application zone towards the fluid absorption zone
13 causes analyte antibodies and cross-reacting
14 antibodies to first contact the preabsorbing
15 groups and subsequently the analyte antigens.
- 16 3. Contacting the membrane with a suitably labelled
17 reagent capable of binding to any analyte
18 antibodies bound to the membrane by complexation
19 with their respective analyte antigens and
20 indicating the presence of such bound antibodies
21 such indicia including but not being limited to
- 22 • Deposition of a visually discernible moiety at the
23 site of antibody binding
 - 24 • Deposition of a fluorescent moiety at the site of
25 antibody binding such moiety being visualised
26 either by fluorescence spectrometry or visually
27 upon application of light at the wavelength
28 necessary to cause fluorescence of the fluorophore
29 employed
 - 30 • Production of a visually discernible, fluorescent
31 or chemiluminescent product at the site of

1 antibody binding by the action of a catalytic
2 moiety attached to the labelled reagent which is
3 used to detect bound antibodies and which is
4 subsequently exposed to a suitable substrate
5 wherein said visually discernible, fluorescent or
6 chemiluminescent product is generated by the
7 action of the catalytic moiety on the substrate.

8

9 A preferred embodiment of the invention may comprise

10

- 11 1. Forming an assay for the detection of antibodies
12 specific to Herpes simplex virus type 2 (HSV-2)
13 which may be present in a patient sample such
14 assay being composed of a membrane capable of
15 conducting fluid flow and incorporating a sample
16 application zone, a fluid absorbent zone, a line
17 of antigen derived from Herpes simplex virus type
18 1 (HSV-1) and a line of antigen derived from HSV-2
19 such that the lines of HSV-1 and HSV-2 derived
20 antigens are interposed between the sample
21 application zone and the fluid absorption zone and
22 the line of antigen derived from HSV-2 is
23 interposed between the line of antigen derived
24 from HSV-1 and the fluid absorption zone. Lines of
25 immobilised antigens are applied to the membrane
26 such that lateral fluid flow along the membrane
27 causes all components present in the fluid to
28 contact the lines of immobilised antigens.
- 29 2. Applying a whole blood sample to the sample
30 application zone on the membrane such that fluid
31 flow along the membrane by capillary action from

- 1 the sample application zone towards the fluid
2 absorption zone causes antibodies specific for
3 HSV-2 and potentially cross-reacting antibodies to
4 first contact the HSV-1 derived antigen and
5 subsequently the HSV-2 derived antigen.
- 6 3. Contacting the membrane with a detector reagent
7 comprising an anti-human immunoglobulin antibody
8 conjugated to colloidal gold such that binding of
9 the detector reagent to sample derived membrane
10 bound antibodies leads to the deposition of a
11 visibly discernible complex at the site on the
12 membrane where the antibodies are bound.
- 13 4. Determining the presence or absence of antibodies
14 to HSV-2 in the patient sample by visual
15 discernment of the presence or absence of colour
16 at the site of immobilisation on the membrane of
17 the HSV-2 antigen.

18

19 Advantages of the Invention Over Present Technology

20 This invention enables the development of assays
21 which utilise the native members of pairs of SBPs
22 whilst, minimising false results due to the presence
23 of other ligands which might be capable of binding to
24 the member of the pair of SBP utilised in the assay.
25 An additional advantage in the general embodiment
26 described is that no sample pre-treatment is required
27 to effect this improvement.

28

29 Advantages of the invention in the specific
30 embodiment

1 The detection of type specific antibodies to Herpes
2 simplex virus type 2 (HSV-2) in a patient sample is
3 complicated by the close antigenic similarity between
4 HSV-2 and HSV-1, the latter very commonly occurring
5 in most populations (60-100% prevalence in adult
6 populations). The detection of type specific
7 antibodies involves utilisation of type specific
8 antigens, however, there are no antigens either in
9 HSV-1 or HSV-2 which are totally unique to the
10 individual type. Even antigens which are generally
11 considered to be type specific (for example
12 glycoprotein G) show a degree of similarity. For
13 this reason, several attempts have been made to
14 construct type specific serological tests for HSV-2
15 which utilise regions of certain HSV-2 antigens which
16 are truly unique to the individual type. Such
17 antigen fragments may be constructed using
18 recombinant gene technology or as synthetic peptides.
19 The problem with such antigens is that they may not
20 accurately or completely represent the full range of
21 epitopes presented to a patient by the antigen in its
22 native state (i.e. during the course of an
23 infection). Therefore, such antigens may not bind
24 certain patient antibodies produced in response to an
25 infection and as such, lead to assays of reduced
26 sensitivity.

27
28 The advantage of the invention in this specific
29 embodiment is that it enable the manufacture of
30 serological tests for antibodies to HSV-2 which
31 utilise a truly native antigen (i.e. the protein

1 purified from the HSV-2 organism) whilst removing
2 those potentially cross-reactive antibodies derived
3 from an HSV-1 infection which could compromise the
4 specificity of such an assay.

5
6 Use of the present invention will make the results of
7 assays more reliable by significantly reducing the
8 occurrence of false results due to the presence of
9 other ligands which might be capable of binding to
10 the member of the pair of SBP utilised in the assay.
11 Thus diagnoses based on the results of such tests
12 would also be more reliable.

13
14 In practice, the following points should also be
15 accounted for.

16 • In the specific embodiment of the invention
17 described above, a design specification shall be
18 written which shall ideally but not exclusively
19 describe the use of potentially cross-reacting
20 antigens to improve the specificity of a
21 serological assay for antibodies to herpes simplex
22 type 2 (HSV-2). This shall involve

- 23 1. A permeable membrane strip which incorporates a
24 sample application zone, lines of HSV-1 and
25 HSV-2 antigen and anti-human immunoglobulin G
26 antibody and a zone of an absorbent capable of
27 absorbing all the liquid applied to membrane.
- 28 2. The strip is housed in a casing of suitable
29 material with apertures to enable the
30 application of a sample to the sample
31 application zone, the visualisation of the

1 membrane in the region of the HSV-2 antigen and
2 anti-human antibody and the application of a
3 detector reagent such that the sample
4 application aperture is between the detector
5 reagent aperture and the visualisation
6 aperture.

7 3. The antigen lines, antibody line and absorbent
8 zone are laterally spaced such that a sample
9 applied in the sample application zone first
10 contacts the HSV-1 antigen, then the HSV-2
11 antigen, then the anti-human antibody, then the
12 absorbent zone as it diffuses along the
13 membrane.

14 4. A detector reagent comprising a colloidal gold
15 labelled anti-human immunoglobulin antibody
16 capable of binding to antibodies which bind to
17 the immobilised HSV-2 antigen and to the anti-
18 human antibody is then added to the membrane at
19 the detector reagent aperture.

20 5. The labelled detector reagent will then diffuse
21 along the membrane and bind to immobilised
22 antibodies bound to the HSV-2 antigen and anti-
23 human antibody thereby enabling visualisation
24 of the bound antibodies in the visualisation
25 aperture of the device.

26

27 Development of the technology and application of this
28 invention will centre around the following areas;
29 Further prototype devices based upon this design will
30 be constructed and the diagnostic performance
31 verified, manufacturing product specifications shall

1 be developed. Product will be manufactured according
2 to the manufacturing specifications. The product
3 will be evaluated in clinical trials in appropriate
4 target populations. The product shall be placed into
5 the market.

6
7 A general embodiment of the invention given above
8 describes the use of the invention in a test to
9 detect antibodies to specific antigens. Since the
10 detector reagent will also detect antibodies which
11 bind to the preabsorbing antigen or antigens, the
12 invention also relates to an embodiment wherein the
13 preabsorbing antigen may be placed such that it is
14 visible in the visualisation window of the device and
15 the test separately measure antibodies to these other
16 antigens. In addition, the order of spacing of the
17 various binding moieties on the membrane indicates
18 whether a binding moiety is being used as a
19 preabsorbing group. For example, in a specific
20 embodiment of the invention given above which
21 describes the use of the invention in a test to
22 detect specific antibodies to HSV-2, it would be
23 possible to construct a test utilising a line of HSV-
24 1 antigen to preabsorb cross-reacting antibodies and
25 thereby improve the specificity of the HSV-2 test.
26 However, if the HSV-1 line were placed between the
27 sample application zone and the HSV-2 line then its
28 function would include preabsorbing potentially
29 cross-reactive antibodies to improve the performance
30 of the HSV-2 test.

31

1 In specifying the nature of the preabsorbing moiety
2 (eg. natural source molecule, recombinant protein),
3 it is possible to utilise a number of technologies
4 for developing alternative preabsorbing group mimics
5 (eg. anti-idiotypic antibodies, non-peptide
6 mimetics). The nature of the preabsorbing groups need
7 not be specified.

8
9 An example of the invention is illustrated in the
10 following example and with reference to the
11 accompanying Figure.

12
13 Figure 1 illustrates diagrammatically a nonlimiting
14 device according to the invention.

15
16 Example:

17
18 Preabsorbing antigen, Herpes Simplex Virus type 1
19 (HSV-1) recombinant glycoprotein G, 0.2ul/cm (Biokit,
20 Spain), were printed on to nitrocellulose (Whatman
21 Immunopore 5.0 u) membrane strips (0.5 x 20mm)
22 supported on polyethylene strips in a fashion well
23 known to those skilled in the art. Test antigen,
24 partially purified native Herpes simplex Virus Type 2
25 (HSV-2), 40ng/cm protein (Biokit, Spain), were
26 printed downstream of this line in relation to liquid
27 flow along the membrane. The membrane was dried,
28 blocked with a solution containing sucrose (4%), BSA
29 (0.3%) and Tween (0.01%) and dried.

30

1 A pad (conjugate pad), 0.5 x 10mm, (Millipore Quick
2 Release) was impregnated with a solution containing
3 polyvinylalcohol, BSA and Triton. After drying, the
4 pad was sprayed with a solution containing goat anti
5 human IgG(Sigma) conjugated to gold sol (prepared by
6 a method known by those skilled in the art). After
7 drying, the processed pad was attached to the
8 membrane (see Figure 1).

9

10 A liquid absorbing pad (absorbent pad) 0.5 x 20mm
11 (S&S) was placed on the membrane (see Figure 1).

12

13 A second liquid absorbing pad 0.5 x 20mm (S&S) was
14 placed in contact with the conjugate pad (see Figure
15 1).

16

17 A 20ul sample of human serum was added to the
18 membrane between the conjugate pad and the
19 preabsorbing antigen. The sample moved laterally
20 sequentially across the Preabsorbing antigen line,
21 Test antigen line, and the Control line. After 30
22 seconds, buffer, 150ul PBS, was added to the buffer
23 pad. The liquid moved onto the conjugate pad and
24 releases the goat anti human IgG-gold conjugate on to
25 the membrane. The conjugate moved laterally
26 sequentially across the membrane, passing through the
27 Preabsorbing antigen line, Test antigen line, and the
28 Control line and then onto the absorption pad.

29

30 If the sample contained IgG antibodies to HSV-2,
31 these were complexed with the Test gG2 antigen.

1 Potentially cross contaminating, HSV-1 IgG antibodies
2 to gG1, were complexed with the Preabsorbing antigen.
3 Residual IgG antibodies in the sample were complexed
4 with the Control line. If IgG antibodies are
5 complexed at the Preabsorbing antigen line, Test
6 antigen line, and the Control line then these will
7 react with the anti-human IgG-gold conjugate yielding
8 a pink/red line.

9
10 Serum samples containing IgG antibodies to HSV-1 and
11 HSV-2 glycoprotein G were assayed with the test
12 device. Coloured lines were observed at the
13 Preabsorbing antigen, Test antigen, and Control
14 respectively.

15
16 Serum samples containing only HSV-1 IgG antibodies
17 yielded only two coloured lines Preabsorbing antigen,
18 and the Control lines respectively i.e. a negative
19 test for HSV-2 antibody result.

20
21 If devices were manufactured omitting the
22 preabsorbing antigens and these devices were used to
23 assay serum samples containing only HSV-1 IgG
24 antibodies, some devices yielded two coloured lines,
25 Preabsorbtion, Test and Control, i.e. a false
26 positive result.

27
28 In Figure 1 the labels represent:

- 29 1. Preabsorbing Antigen
30 2. Test Antigen
31 3. Control

- | | | |
|---|----|-----------------|
| 1 | 4. | Membrane |
| 2 | 5. | Conjugate Pad |
| 3 | 6. | Buffer Pad |
| 4 | 7. | Absorption Pad. |

1 **CLAIMS**

- 2
- 3 1. An assay device capable of detecting an analyte
- 4 which is a member of a pair of specific binding
- 5 partners, the device comprising a preabsorbing
- 6 zone comprising at least one immobilised antigen
- 7 and/or receptor moiety which function as pre
- 8 absorbing group(s) and a specific binding zone
- 9 wherein a sample suspected of containing the
- 10 analyte passes through the preabsorbing zone
- 11 prior to entering the specific binding zone.
- 12
- 13 2. A device as claimed in claim 1 which is a
- 14 membrane based device capable of conducting
- 15 fluid flow.
- 16
- 17 3. A device as claimed in claims 1 or 2 comprising
- 18 a sample application zone, a fluid absorbent
- 19 zone, at least one line of immobilised
- 20 preabsorbing groups and a specific binding zone.
- 21
- 22 4. A device as claimed in claim 3 wherein at least
- 23 one line of preabsorbing groups is interposed
- 24 between the sample application zone and the
- 25 fluid absorption zone.
- 26
- 27 5. A device as claimed in any of the preceding
- 28 claims wherein sample movement is facilitated by
- 29 capillary action.
- 30

- 1 6. A device as claimed in any of the preceding
2 claims wherein the sample is selected from the
3 group consisting of whole blood, serum, plasma,
4 interstitial fluid, semen, senunel plasma, urine
5 and saliva.
6
- 7 7. Use of a device as claimed in any of the
8 preceding claims to detect analyte antibodies by
9 complexation with labelled analyte antigens.
10
- 11 8. Use of device as claimed in any of claims 1 to 6
12 to detect analyte antigens by complexation with
13 labelled analyte antibodies.
14
- 15 9. Use of a device as claimed in any of claims 1 to
16 7 for the detection of antibodies specific to
17 Herpes simplex virus type 2.
18
- 19 10. A method of detecting an analyte which is a
20 member of a pair of specific binding partners in
21 a sample wherein the sample suspected of
22 containing the analyte is exposed to at least
23 one preabsorbing moiety before being exposed to
24 the specific binding partner of the analyte.

1/1

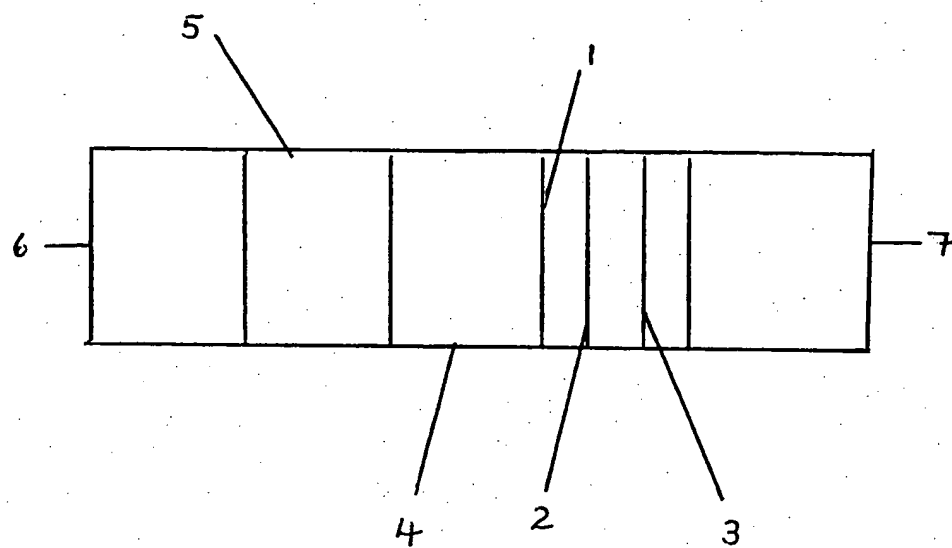


Fig. 1